Random Coil–β-Form Transition of Poly-L-lysine. Evidence for the Formation of the α-Helical Structure during the Transition

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Summary By c.d. measurements and potentiometric titration experiments it has been shown that the pH-induced coil–β-form transition of poly-L-lysine at 50° occurs through the formation of the right-handed α-helical structure.

IT is well known that uncharged poly-L-lysine $(\text{L-Lys})_n$ assumes in aqueous solution either the α-helical conformation or the β-structure depending on temperature conditions.[1] Recently, potentiometric and microcalorimetric experiments were carried out by different research groups to determine the thermodynamic parameters relative to the conformational transition from an uncharged random coil to an uncharged β-structure. The standard enthalpy change ΔH_c^0 at 25° was 870 cal mol^{-1} from potentiometry,[2] and -1300 cal mol^{-1} from microcalorimetry.[3] This difference is unacceptably large and we have undertaken a careful re-investigation of this problem using both experimental techniques. During our potentiometric experiments, carried out at 40°, 50°, 60°, and 70°, we found that the time required for the pH to reach the equilibrium value after each addition of titrant was much higher than that reported by other investigators and inversely proportional to the temperature. Hermans and his co-workers[2] ascribed such behaviour to the slow formation of the β-structure. We have been able to show that at 50° this conformation forms so slowly that the thermodynamically unfavoured α-helical conformation forms first, and subsequently there is a slow conversion to the β-structure. To a dilute solution of $(\text{L-Lys})_n\cdot\text{HCl}$ (0·5 mg/ml) at 50° an amount of 1N KOH, necessary to reach a degree of ionization α of 0·8 (80% of ε-amino groups deprotonated) was quickly added. C.d. measurements were carried out on some of the solution with a Cary 6001 c.d. instrument equipped with a thermostatable cell assembly. The pH of the remaining solution was measured as a function of the time from KOH addition. The results are shown in Figure 1. The c.d. spectrum recorded 15 minutes after the addition of titrant shows that the polymer conformation is a mixture of α-helix and random coil.[4] Subsequently the fraction of the polymer in α-helical form undergoes a very slow transition to the β-structure, as indicated by the blue shift and by the decreased intensity of both bands. The final spectrum (curve 6) is consistent with a mixture of β and random coil conformations.[4] During the α-helix–β-structure transition (which takes several hours at 50°) the pH of the solution decreases continuously, reaching the final value when no more α-helical form is present in the system.

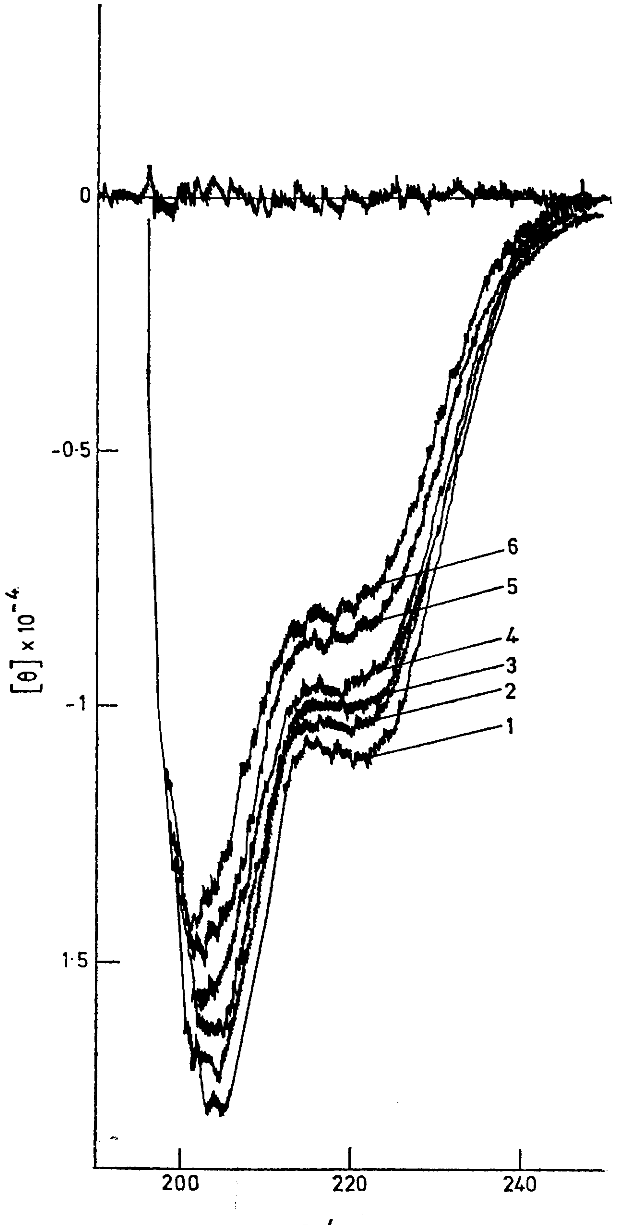

FIGURE 1. Original c.d. spectra of (L-Lys)$_n$ in aqueous solution at α = 0·8, as a function of the time from the addition of titrant. Curves 1, 2, 3, 4, 5, and 6 after 15, 50, 90, 230, 390, and 530 min respectively. All spectra have been recorded with a Cary 60 instrument equipped with c.d. attachment and thermostatable cell assembly. In both figures the ordinate scale has been expressed in molar ellipticity units, taking into account the molar concentration of lysine residues and the cell path-length.

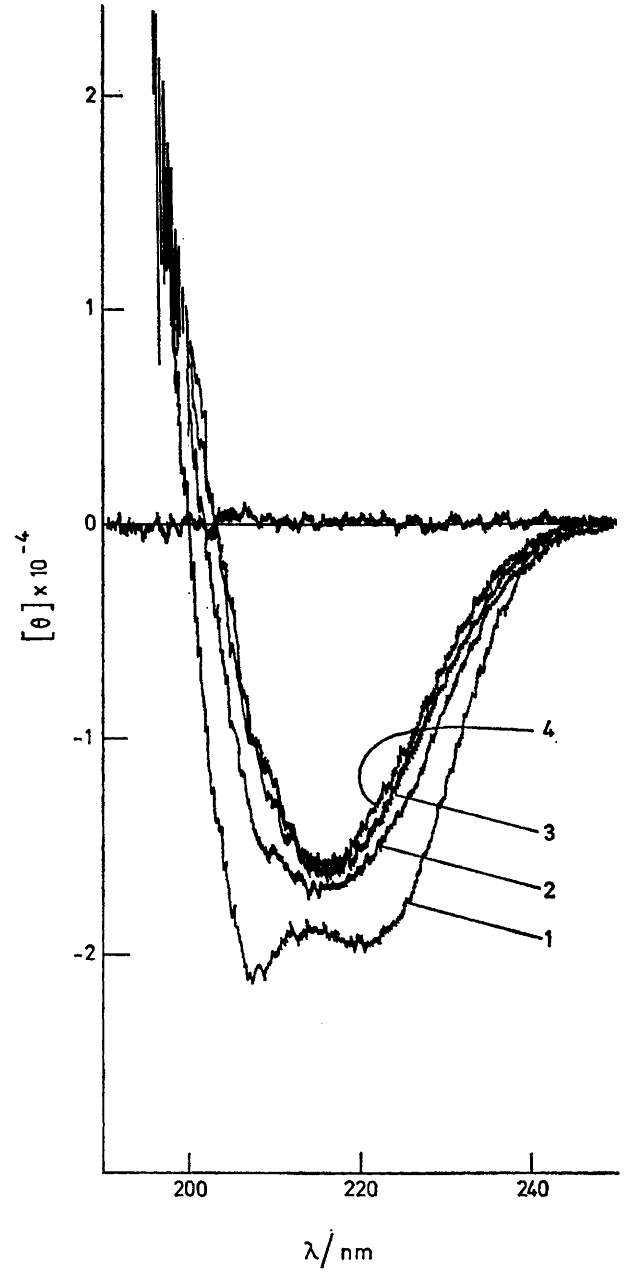

FIGURE 2. Original c.d. spectra of (L-Lys)$_n$ in aqueous solution at α = 1 as a function of the time from the addition of titrant. Curves 1, 2, 3, and 4, immediately after addition and after 10, 22, and 32 min respectively.

The same experiment has been performed on completely deprotonated (L-Lys)$_n$ at 50° and the c.d. spectrum was recorded immediately (Figure 2). The spectrum is characterized by two negative bands at 222 nm and 208 nm with molar ellipticity values $[\theta]$ of $-19{\cdot}500$ and $-21{\cdot}400$ respectively. By comparison with computed c.d. curves of (L-Lys)$_n$ containing varying amounts of α-helix, β-form, and random coil structures[4] it seems that this pattern corresponds to a mixture of random coil and α-helical conformations containing approximately 40% of random coil. Subsequently there is the transition to the β-structure. After 40 minutes the whole polymer assumes completely the β-conformation, the rate of the transition being much higher than at $\alpha = 0{\cdot}8$. Also in this case the decrease of the pH parallels the formation of the β-structure. During this experiment no polymer precipitation was observed.

Obviously, reliable thermodynamic data on the coil–β-form transition of (L-Lys)$_n$ can be obtained from potentiometric data provided that the experimental pH values refer to an equilibrium system containing random coil and β-form only. Our results show that this condition can be achieved only by waiting much longer than previously reported after the addition of titrant.

From potentiometric titration experiments carried out using the precautions suggested by the c.d. results, we obtained $\Delta H^0_c = 130 \pm 50$ cal mol^{-1} for the conformational transition, unchanged coil–uncharged β-form. This value is lower than that reported by Hermans,[2] and still in disagreement in magnitude and sign with respect to that obtained by Scheraga in a single calorimetric experiment.[3] It is also worth noting that at 50° we found a ΔG^0_c value of -130 cal mol^{-1} for the coil–β-structure transition. This value is about 100 cal mol^{-1} more negative than that obtained extrapolated at 50° from the data of Hermans[5] for the coil–α-helix transition (*c.a.* -10 cal mol^{-1}). However, in spite of the unfavourable thermodynamic factors, the α-helical conformation forms first with respect to the β-structure, due to kinetic reasons.

More detailed results on the thermodynamic aspects of the coil–β-structure transition of (L-Lys)$_n$ will be reported elsewhere.

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